COMPARISON OF THREE METHODS OF DETECTION FOR GROUP A STREP PHARYNGITIS: **ILLUMI**GENE® GROUP A *STREPTOCOCCUS* DNA AMPLIFICATION ASSAY, OSOM® ULTRA STREP A TEST, AND ROUTINE BACTERIOLOGIC CULTURE

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Rheumatic fever and rheumatic heart disease have been on the decline in the last century, but several episodes of resurgence in the United States and Europe, as well as continued high mortality in developing countries, reinforce the need for continued development of improved methods for detection of the causative agent, Group A Strep pharyngitis. Traditionally diagnosed via clinical and bacteriologic methods, newer molecular strategies to identify Group A Strep in patient samples have become available, but their acceptance for clinical testing has been thwarted by test complexity, expense, and lack of timeliness. With the advent of LAMP molecular technology, simpler molecular tests more appropriate for rapid, inexpensive diagnostic assays are becoming available. A comparison of one of these assays, **illumi**gene® Group A *Streptococcus* DNA Amplification Assay, with traditional rapid antigen and culture testing from the perspective of sensitivity, specificity, accuracy, convenience and ease of use, time constraints, "real" value, process improvement, and cost was undertaken. **illumi**gene® outperformed combined antigen and culture testing in sensitivity, accuracy, and turnaround time. Both methodologies were very similar for specificity and perceived test convenience. In a data review from the perspective of diagnostic accuracy, **illumi**gene® was 99.5% accurate compared to combined accuracy rate of 93.2% for antigen and culture testing, indicating that one case would have been misdiagnosed by **illumi**gene® compared to 36 cases being misdiagnosed or treatment delayed using traditional testing

methods. Three different approaches to cost comparison yielded equivocal results that cannot be resolved without further real-world study, but **illumi**gene® outperformed routine antigen and culture testing in two of three cost scenarios. Based on the data obtained in this study, **illumi**gene® has proven to be a viable contender for inclusion in future algorithms for improved diagnosis of Group A Strep pharyngitis, and its use will mitigate the damaging effects of this disease and its sequelae.

To the many who kindled the spark of curiosity that led me down this path.

I wouldn’t be here without your encouragement.

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INTRODUCTION

Rheumatic fever is a delayed autoimmune response to pharyngeal infection caused by *Streptococcus pyogenes*, commonly known as Group A Strep. A single untreated case of Group A Strep pharyngitis puts a person at risk for this lifelong, destructive, chronic disease. Major clinical symptoms of rheumatic fever can include heart murmur, joint swelling and tenderness, and chorea. Rheumatic fever, along with its chronic form rheumatic heart disease, has long been a public health problem worldwide, and while the number of cases in industrialized nations had been decreasing since the mid-20th Century, incidence has increased in less developed nations during the same period, especially in young adults. In 1994, there were 12 million people worldwide afflicted with rheumatic fever and rheumatic heart disease. In 2000, there were an estimated 332,000 deaths in children and young adults globally (World Health Organization [WHO], 2004). Recurrent outbreaks of rheumatic fever in the United States that have occurred without warning or explanation (Veasy et al., 1987) have negated the maxim that rheumatic fever is present only in developing countries, and it is recognized that rheumatic fever remains an unconquered, increasingly serious global issue.

Rheumatic fever and its chronic form rheumatic heart disease, arise specifically and exclusively from infection with Group A Strep pharyngitis. In the United States alone, 28–36 million throat cultures are obtained annually for sore throat. It is estimate that most children worldwide experience at least one episode of pharyngitis per year, 15–

20% of which are caused by *Streptococcus pyogenes,* the most common cause of bacterial pharyngitis (Mahon, Lehman, & Manuselis, 2011). Surveys of healthy school children ages 6–10 years found antistreptolysin O titers, indicative of production of antibody to streptococcal infection, in 15–70% of children, beta-hemolytic streptococcal carrier rates of 10–50% in asymptomatic school children, and in temperate climates, 50–60% of streptococci isolated from asymptomatic children to belong to serogroup A (WHO, 2004). In the last 20 years, rates of *Streptococcus pyogenes* pharyngitis and asymptomatic carrier states have remained stable, but increased virulence and severity of infection have been documented (WHO, 2004; Veasy et al., 1987). These studies confirm the continued pathogenicity of this organism worldwide and underscore the need for further evaluation and control of the bacterium in human populations that clearly continue to undergo exposure to and infection with this microorganism.

The primary symptoms associated with Group A Strep pharyngitis are tenderness and enlargement of cervical lymph nodes, tonsillar or pharyngeal exudate, pain on swallowing, and fever (Dos Santos & Berezin, 2005). Similar symptoms, with the addition of cough, conjunctivitis and coryza, are more indicative of a viral entity. One of the confounders of diagnosis for Group A Strep pharyngitis is that presentation is quite variable depending on age group. For example, children younger than school age usually do not display the pharyngeal exudate or inflammation, and their symptoms are less acute. These children may not be medically evaluated because their parents may believe the child's symptoms do not warrant treatment. Older children and adolescents are more likely to have classical symptoms as well as rash. Symptoms of this bacterial infection are self-limiting and usually abate after about 5 days, even without antibiotic therapy. If

a child has symptoms of Group A Strep pharyngitis and a throat swab is obtained, a negative result for Group A Strep traditionally prompts culture, which takes 24–48 hours for definitive identification. Antibiotics begun within 9 days of onset of symptoms of Group A Strep pharyngitis usually will prevent later kidney, cardiac, joint, or neurological sequelae (American Academy of Pediatrics, 2009). Conversely, receiving no antibiotic treatment, even though symptoms have abated, can result in rheumatic fever or other sequelae weeks after the initial throat infection. Factors to be considered in diagnosis of Group A Strep pharyngitis include age group of patient, country, season, socioeconomic conditions, exposure to someone with the infection, crowded living conditions, other environmental factors, genetic predisposition of host, quality and availability of health care, and education about the infection and health care recommendations, as well as infection level in the community.

While a diagnosis of Group A Strep pharyngitis may appear straightforward, there is evidence that accurate clinical identification of this bacterial infection is more difficult than expected. In a study from 2005, children with sore throats were evaluated by physicians, who were asked, “Would you treat this child with antibiotics?” Physician recommendations were based entirely on clinical findings. Each case was subsequently evaluated with microbiological testing. Physicians in this study would have treated 47% of the Group A Strep-negative children and would not have treated 21% of the Group A Strep-positive cases. This study (Dos Santos & Berezin, 2005) lends credence to the judgment that a specific algorithm for diagnosis of Group A Strep pharyngitis, which includes both clinical and laboratory protocols, would improve patient outcomes. Nevertheless, diagnostic protocols continue to be quite variable (Facklam, 1976; Kellogg,

1990). A study reviewing 12 national guidelines for diagnosis of Group A Strep pharyngitis (Chiappini et al., 2011) showed that in some European nations, sore throat is believed to be a common malady, "self limiting and benign," that should be treated symptomatically only, and throat culture should be done only in very specific cases. In the United Kingdom, Scotland, Holland, and Belgium, rapid antigen detection tests from swabs are not recommended; rather, diagnosis of Group A Strep pharyngitis is made clinically and epidemiologically. In Canada, patients at risk for Group A Strep pharyngitis undergo throat culture but not rapid antigen testing. Even in the United States, there is disagreement among protocols, with guidelines differing based on which professional group is making the recommendations. For example, the American Society of Internal Medicine recommends culture only in children with appropriate Centor scores (Centor, Allison & Cohen, 2007; Tanz et al., 2009). The American Heart Association and American Association of Pediatrics recommend evaluation of history, clinical findings and rapid antigen detection testing in all cases of suspected Group A Strep pharyngitis. If rapid antigen is negative, the sample should reflex to routine 24–48 hour microbiological culture for definitive diagnosis, especially in children. This is currently the gold standard for diagnosis of *Streptococcus pyogenes* pharyngitis. A direct antigen test can give results in minutes right in the health care provider's office, but sensitivity of this test can average from 70% to 95%. As recently as 2008, significant discrepancy between rapid antigen detection testing and culture was documented, with sensitivity of rapid antigen at 70% and that of in-office culture 81% (Tanz et al., 2009). These data imply that a large number of patients with Group A Strep pharyngitis remain untreated. Sensitivity of rapid antigen detection testing improved with Centor scores in this review.

Newer technologies for diagnosis of Group A Strep pharyngitis have become available, such as fluorescence in situ hybridization and other nucleic acid probe methods, but these assays have proven no more sensitive than culture, and most remain costly and time-consuming, involving much hands-on processing (Ding & Wang, 2011). Rapid polymerase chain reaction (PCR) technology has a sensitivity equal to that of culture, with specificity of 98%, and could replace traditional culture, save for the cost of the technology (Slinger et al., 2011, Santos et al., 2003). Newer molecular assays employ some of the principles of PCR, but avoid some of its pitfalls. One such test is the **illumi**gene® assay, a nucleic acid amplification procedure that is free from some of the time and temperature constraints of traditional PCR (Meridian Bioscience, 2012).

The goal of this thesis is to compare three specific test methodologies for the diagnosis of Group A Strep pharyngitis with respect to sensitivity, specificity, accuracy, ease of use, convenience for operators, time constraints, real value, process improvement and cost, and to demonstrate data supportive of modification in current diagnostic algorithms. OSOM® Ultra Strep A Test (Sekisui Diagnostics, Lexington, MA), **illumi**gene® Group A Streptococcus DNA Amplification Assay (Meridian Bioscience, Cincinnati, OH), and routine bacteriologic culture are the methods to be compared.

OSOM® Ultra Strep A Test is a Clinical Laboratory Improvement Amendments (CLIA)-waived color immunochromatographic assay for the rapid qualitative detection of Group A Strep antigen directly from throat swabs. Viable and nonviable organisms can be detected within 7 minutes. The test strip, a lateral flow device, allows antibody to capture Group A Strep antigen if it is present and produce a visually detectable colorimetric change via capillary action and complex capture. See Appendix A.

The **illumi**gene® Group A *Streptococcus* DNA Amplification Assay is a qualitative test for Group A Strep antigen obtained directly from throat swabs. This method employs a loop-mediated isothermal DNA amplification format (LAMP). See Appendix B. In the case of Group A Strep, this assay is designed to seek a segment of the Group A Strep SpeB gene. If this gene segment is identified, it is amplified, the detectable end-point of which is increased turbidity of the sample, a positive reaction. No change in turbidity implies that no Group A Strep DNA is present. See Appendix C.

A more specific diagnostic algorithm with emphasis on astute clinical diagnosis, rapid laboratory confirmation, and prompt and proper antibiotic treatment of Group A Strep is critical for control of rheumatic fever and other sequelae of Group A Strep infection. It is the hope of this writer that the observations herein will promote adoption of new diagnostic methods for Group A Strep pharyngitis, with the ultimate goal of improved patient outcomes regarding this affliction.

METHODS

Throat swabs from Primary Children's Medical Center in Salt Lake City, Utah, were obtained per the American Academy of Pediatrics standard of care (American Academy of Pediatrics, 2009) and at physician discretion. Two throat swabs were obtained per person. The first swab was tested with the OSOM® Ultra Strep A Test. The second swab was used for routine culture per standard microbiology laboratory protocols (Intermountain Health Care [IHC] Laboratory Services, 1999; IHC Laboratory Services, 2006). This second swab was then refrigerated and subsequently used for molecular testing on the **illumi**gene® Group A *Streptococcus* Assay for detection of Group A Strep DNA. Testing was conducted over a 62-day interval in the autumn of 2012. All patient samples were submitted with a physician-directed order for Group A Strep testing, with the presumption that these patients had presented with signs and symptoms of Group A Strep pharyngitis (WHO, 2004). A total of 368 dual-swab samples were tested with the three methods. All swabs tested with the OSOM® Ultra Strep A Test for rapid antigen detection were processed in accordance with the procedural recommendations outlined in the package insert (Sekisui Diagnostics, 2012). All swabs earmarked for testing with **illumi**gene® were processed within 6 days of receipt in the microbiology laboratory and in accordance with the procedural instructions in the **illumi**gene® Group A Streptococcus Assay package insert (Meridian Bioscience, 2012). Controls and system validations were also performed in accordance with procedural recommendations outlined in both package

inserts.

Comparison of sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the different test methods was performed. In addition, factors relating to convenience of use and interpretation, cost, process improvement, and clinical value of the test protocols were evaluated.

At our facility, the routine protocol for testing of throat swabs is to conduct OSOM® Ultra Strep A Test antigen screening within 1 hour of swab receipt, followed by routine 24–48 hour culture for clear-cut identification. In this pediatric population, negative antigen testing always reflexes to culture for definitive diagnosis. Since culture is still the traditional method for Group A Strep identification (Langlois & Andreae, 2011), culture was the gold standard on initial testing in this study. Discrepant results between antigen testing, routine culture, and **illumi**gene® were subsequently evaluated using an alternate nucleic acid amplification method targeting a nucleic acid sequence of the *Streptococcus pyogenes* SpeB gene that differed from the sequence sought in the **illumi**gene® assay. This nucleic acid amplification procedure was used as our gold standard in the discrepancy testing portion of this study. Since antigen testing and culture were both performed for each case as part of our facility's protocol, data collected in this study were evaluated in one of two ways: (1) either as three stand-alone tests being compared to each other or (2) OSOM® and culture as one test being compared to **illumi**gene®. Since the testing protocol for Group A Strep pharyngitis at our facility requires that rapid antigen detection test results be reported within 1 hour of swab receipt, a comparison was made between turnaround time for OSOM® (J.A. Daly, personal communication, January 31, 2013) and routine culture and **illumi**gene® to see if

illumigene® could provide the 1-hour turnaround time required.

The perceived convenience of the **illumi**gene® procedure from the perspective of the test operators, comparing it to the routine rapid antigen/culture protocol, was assessed. Medical laboratory scientists familiar with both culture and **illumi**gene® were questioned about seven convenience characteristics comparing both methods and ranked the protocols for the amount of training required, time to diagnosis, perceived complexity of the testing and equipment, number of steps required to complete the assay from start to finish, amount of hands-on time required to reach a final result, ease of interpretation for each test, and how often a test had to be repeated to obtain a valid result. Operators ranked the protocols from least convenient (1+) to most convenient (4+). See Appendix D.

Hands-on versus hands-free time for test processing was evaluated for the three methods.

Comparison of the cost for rapid antigen/routine culture testing versus that for the **illumi**gene® molecular assay was performed as a part of this evaluation. Several different cost perspectives were included to help clarify the real costs of these tests since actual test expenses and reimbursement are exceedingly variable and difficult to pinpoint. Raw data from our study data were evaluated with "cost" defined as time spent by clinical laboratory assistants and medical laboratory technologists per case (Bureau of Labor, 2013; Medical Laboratory Observer, 2013) plus material cost per case (J.A. Daly, personal communication, March, 2013). A sampling of allowable reimbursement rates was also obtained to compare to the overall cost of the different test strategies (J.A. Daly, personal communication, January, 2014). Meridian Bioscience financial projection data

were reviewed. In addition, information relating to actual cost of tests and allowable reimbursement amounts was obtained to compare to the costs we established for tests based on our data (James, 2014).

The value of a laboratory assay, in the view of health care providers (A.J. Blaschke, personal communication, July 12, 2013), is its ability to provide a dependable answer about a patient sample within a reasonable length of time. When clinicians submit specimens for testing, they want the results to be accurate and trustworthy. Thus, a retrospective evaluation of the data accumulated in this study was performed to compare OSOM®, culture and **illumi**gene® in a simulated laboratory setting as if all three methods were routinely employed on all throat swabs suspected of containing Group A Strep. The goal was to compare the value, as defined above, of each of these tests either as stand-alone assays or in combination to see which would be the most beneficial to patients. Discrepancy testing data were used to clarify any initially equivocal test results.

RESULTS

Using culture as the gold standard, initial sensitivity for OSOM® rapid antigen testing was 84.2% and specificity was 95.1%. Sensitivity for **illumi**gene® was 97.6% and specificity 91.0%. Positive predictive value (PPV) for OSOM® testing was 83.1% and for **illumi**gene® 76.7%. Negative predictive value (NPV) for OSOM® testing was 95.4% and for **illumi**gene® 99.2%. Overall accuracy for initial OSOM® testing was 92.7% while that for **illumi**gene® was 92.9%. See Table 1 for details. Positivity rate for the presence of Group A Strep was 22.6% (83/368) for OSOM®, 22.3% (82/368) for culture, and 28.3% (104/368) for **illumi**gene® testing.

Table 1 also illustrates results of discrepant resolution testing, which was performed using the alternate nucleic acid amplification method as our gold standard. Postdiscrepant resolution data showed sensitivity for OSOM® testing to be 73.1%, culture 76.9%, and **illumi**gene® 99.0%. Specificity values postresolution were 97.3% for OSOM®, 99.2% for culture, and 99.6% for **illumi**gene®. PPV was 91.6% for OSOM®, 97.6% for culture, and 99.0% for **illumi**gene®, while NPV was 90.2%, 91.6%, and 99.6%, respectively. Overall accuracy for OSOM® testing was 90.5%, while that of culture was 92.9% and **illumi**gene® 99.5%.

As mentioned above, after discrepancy testing, 28.3% (104/368) of all cases submitted for testing were confirmed to be positive for Group A Strep. Of these, OSOM® antigen testing missed 20% (21 of 104) of the positive cases. In the 26

Table 1: OSOM®, Culture, and **illumi**gene®: Pre- and Postdiscrepant Resolution

OSOM®

OSOM® Antigen	Culture (Predis-crepant Resolution)		Total	Postdiscrepant Resolution		Total
	Positive	Negative		Positive	Negative	
Positive	69	14	83	76	7	83
Negative	13	272	285	28	257	285
Total	82	286	368	104	264	368
Sensitivity	84.2%	69/82		73.1%	76/104	
Specificity	95.1%	272/285		97.3%	257/264	
PPV	83.1%	69/83		91.6%	76/84	
NPV	95.4%	272/285		90.2%	257/286	
Accuracy	92.7%	341/368		90.5%	333/368	

Illumigene®

illumigene®	Culture (Predis-crepant Resolution		Total	Postdiscrepant Resolution		Total
	Positive	Negative		Positive	Negative	
Positive	80	24	104	103	1	104
Negative	2	262	264	1	263	264
Total	82	286	368	104	264	368
Sensitivity	97.6%	80/82		99.0%	103/104	
Specificity	91.0%	262/286		99.6%	263/264	
PPV	76.9%	80/104		99.0%	103/104	
NPV	99.2%	262/264		99.6%	263/264	
Accuracy	92.9%	342/368		99.5%	366/368	

Culture

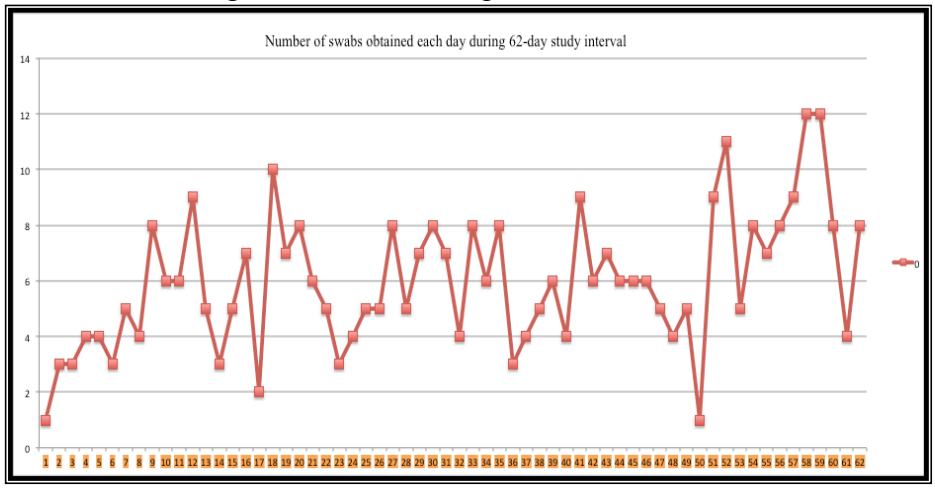
Culture	Prediscrepant Resolution		Total	Postdiscrepant Resolution		Total
				Positive	Negative	
Positive	82		82	80	2	82
Negative		286	286	24	262	286
Total	82	286	368	104	264	368
			Sensitivity	76.9%	80/104	
			Specificity	99.2%	262/264	
			PPV	97.6%	80/82	
			NPV	91.6%	262/286	
			Accuracy	92.9%	342/368	

discrepant cases between routine culture and **illumi**gene®, 23 culture negative cases were confirmed by PCR (and **illumi**gene®) to contain Group A Strep DNA, reflecting that 22% (23 of 104) of positive cases were missed by culture. One culture positive case was misidentified and shown by PCR (and **illumi**gene®) to lack Group A Strep DNA. Only two cases remained discrepant: One **illumi**gene® positive case was culture and PCR negative, representing a false positive rate of less than 0.4% for **illumi**gene®. The other unresolved case was **illumi**gene® negative and culture/PCR positive, representing a false negative rate of less than 1% for **illumi**gene®.

Our facility routinely uses OSOM® as its rapid test of choice to satisfy our 1-hour turnaround time requirement. The **illumi**gene® assay is not currently in use by our facility on a routine basis, so swab receipt information was recorded during the study period in an effort to predict whether **illumi**gene® could be performed within the desired 1-hour time frame and without the need for sample batching. See Figure 1.

As illustrated, distribution of swab receipt at our facility during the study period indicated that a mean of six throat swabs were submitted for Group A Strep testing in a

Figure 1: Swab Receipt Distribution Data



given 24-hour period (range 1–12). In our 62 days of testing, there were only three instances when more than two swabs were submitted within the same 30-minute time period. In each instance, a total of no more than three swabs arrived within the 30-minute interval.

Medical laboratory scientists trained in both **illumi**gene® and routine culture for Group A Strep were asked to compare the two protocols. See Table 2. As far as training to learn the protocols, 25% of respondents ranked **illumi**gene® as more convenient, while 75% saw no difference between **illumi**gene® and culture. All respondents ranked **illumi**gene® as more convenient than culture with respect to time to diagnosis. Half of the respondents ranked culture as requiring fewer steps to diagnosis and being less complicated to perform, while 25% believed **illumi**gene® had fewer steps and was less complicated; 25% saw no difference in this category. Fifty percent of respondents ranked hands-on time for **illumi**gene® to be more convenient than culture, 25% saw no difference, and 25% ranked culture as requiring less hands-on time. All respondents ranked **illumi**gene® as more convenient to interpret than culture. Fifty percent of

Table 2: Operator Perceptions of Convenience: **illumi**gene® Versus Culture

Characteristic	**illumi**gene® more convenient	Culture more convenient	No difference
Training time	25%	---	75%
Time to diagnosis	100%	---	---
Text complexity	25%	50%	25%
Number of steps required	25%	50%	25%
Hands on time required	50%	25%	25%
Ease of interpretation	100%	---	---
Repeats required	25%	50%	25%
Overall	50%	---	50%

respondents ranked culture as requiring fewer repeats to reach diagnosis, 25% ranked **illumi**gene® more convenient in this regard, and 25% saw no difference. Overall, operators were evenly divided as to whether **illumi**gene® or routine culture was more convenient.

Once a swab was received in the clinical lab, an average of 2.25 minutes hands-on and 48.5 minutes hands-free time was required until results were available using the **illumi**gene® assay. In comparison, OSOM® rapid antigen screening test required an average of 2 minutes hands-on and 7 minutes hands-free time, but in this pediatric population reflexed to routine culture for confirmation. Our culture protocol from receipt to identification based on the data set used in this study averaged 12 minutes of hands-on time. Routine culture takes at least 24, and often 48 hours, for definitive diagnosis of Group A Strep, compared to the less than 1 hour receipt-to-result obtainable with both the OSOM® rapid antigen and **illumi**gene® assays. See Table 3.

To assess the value of the **illumi**gene® assay as to "real world" worth from the perspective of health care providers, the raw data obtained in this study were reviewed retrospectively once the final test results were known, as if these samples had been routinely run as part of our usual clinical microbiology laboratory protocol. In our laboratory, throat swab samples suspected of containing Group A Strep are sent for immediate OSOM® rapid antigen screening. In this pediatric population, the screening test, if negative, reflexes to routine culture; however, a rapid screen that is positive for Group A Strep antigen allows the clinician to proceed with antibiotic treatment immediately, while a negative antigen screening test submitted for culture that at 24–48 hours is positive for Group A Strep may delay more immediate treatment with antibiotics.

Table 3: Hands-on and Hands-free Operator Time Associated with Test Methods

Test protocol	Hands-on time	Hands-free time	Time to Diagnosis	Comments
OSOM® Ultra Strep A Test (rapid antigen)	5 minutes	7 minutes	Less than 1 hour	Screen
(A) Rapid antigen/primary culture	8 minutes	24–48 hours	24–48 hours	Diagnostic
(B) Rapid antigen, primary culture, subculture, PYR test	25 minutes	24–48 hours	24–48 hours	Diagnostic
(C) Rapid antigen, primary culture, subculture, PYR test, latex	26 minutes	24–48 hours	24–48 hours	Diagnostic
Illumigene®	2 minutes	49 minutes	Less than 1 hour	Diagnostic

Although the **illumi**gene® assay is not yet routinely used for Group A Strep testing in our laboratory, this retrospective scenario was conducted with the postulation that either **illumi**gene® would be used after antigen screening as the confirmatory assay or would replace both antigen screen and confirmatory culture. See Table 4.

Interestingly, results showed that if only the OSOM® Ultra Strep A Test for antigen screening had been performed on these swab samples, accuracy would have been 90.2% with a 1-hour turnaround time. If only routine culture had been performed on these samples, diagnostic accuracy would have been 92.9% with a 24–48 hour turnaround. If only **illumi**gene® had been performed on these samples, diagnostic accuracy would have been 99.5% with a 1-hour turnaround time. Further, when antigen screening and culture were both used in this scenario, 15.4% (16/104) of cases positive for Group A Strep would have been missed entirely and likely left untreated, and 10.6% of positive cases (11/104) would have had treatment impacted with initiation of

Table 4: Retrospective "What If" Scenario: Routine Clinical Laboratory Testing of 368 Patients Symptomatic for GAS Pharyngitis

Test Protocol	Accuracy	Turn-around Time	Missed Positives	Treatment Effect
OSOM® only	90.2% (333/368)	Less than one hour	28	28 GAS positive patients would not have received antibiotics; 7 unaffected patients would have been given antibiotics
Routine culture only	92.9% (342/368)	24-48 hours	23	23 GAS positive patients would not have received antibiotics; 2 unaffected patients would have been given antibiotics; antibiotic administration for GAS may have been delayed a day or two while awaiting culture results
OSOM® and routine culture	93.2% (343/368)	24-48 hours	16	16 GAS positive patients would not have received antibiotics; 11 would have received antibiotics only after 24-48 hours; 9 unaffected patients would have been given antibiotics
illumi-gene®	99.5%	Less than one hour	1	1 GAS positive patient would not have received antibiotics; 1 unaffected patient would have been given antibiotics

antibiotics a day or two after initial visit because of the length of time required for definitive diagnosis by culture. Finally, 3.4% (9/264) of culture-negative cases would have been misdiagnosed as having Group A Strep pharyngitis and would have been unnecessarily treated in the absence of the organism.

Since OSOM® Ultra Strep A screening test and **illumi**gene® diagnostic testing both support a turnaround time of less than 1 hour, it seems unreasonable to expect that they would both be used for one specimen in our clinical laboratory setting since the preponderance of our cases are from our emergency department or in-house clinics;

however, in the interest of this scenario, which may be relevant to the outpatient setting, where immediate antigen testing may reflex to confirmatory testing in an outside laboratory, if they were both used, screening antigen testing would have been accurate in 92.1% of these cases, with minimal treatment delay possible pending the **illumi**gene® result, which would have been done more rapidly than culture, but would likely take more than 1 hour if the swab had to be transported from a distant outpatient setting for confirmatory testing.

If **illumi**gene® had been the diagnostic test of choice for all of these cases, without use of antigen screening or confirmatory culture, accuracy of the assay would have been, as mentioned, 99.5% with a turnaround time within 1 hour. In all of our testing, only 0.5% of all cases run on **illumi**gene® had to be rerun (2/368), thereby exceeding the 1-hour turnaround time, but the other 99.5% of tests produced a valid result within the 1-hour time frame required by this facility.

"Cost" was evaluated from several different perspectives for purposes of clarifying this less than transparent characteristic of health care. Initially, comparison of average labor and material costs for the two methods (OSOM® antigen/routine culture versus **illumi**gene®) was performed on our raw data. See Table 5. Primary culture procedures varied depending upon the rapid antigen result and the requirements to achieve isolated Group A Strep colonies. Data for the 368 cases were evaluated and each case assigned a cost value. See Table 6. A total of 268 cases that were rapid antigen positive or negative and culture negative required the least testing and were the least expensive (A) at $8 per case (labor and materials). Seventy cases that were antigen positive and culture positive had a midrange cost (B) of $25 per case. Twelve rapid

Table 5. Breakdown of Labor and Material Costs for Testing: Individual Cases

Labor Costs						
Clinical Laboratory Assistant (CLA)			\$24 per hour*			
Medical Laboratory Scientist (MLS)			\$46 per hour*			
Material Costs						
Item	Unit Cost	CLA Cost	MLS Cost	Turna-round Time	Total Cost	Comments
OSOM®	\$2	\$2 (5 minutes)		1 hour	\$4	Screen
Primary plating	\$1	\$1 (1 minute)	\$2 (1–2 minutes)	24–48 hours	\$4	Diagnostic
Subculture/A disk	\$1	---	\$6 (6–7 minutes)	24–48 hours	\$7	Diagnostic
PYR test	\$2	---	\$8 (10 minutes)	24–48 hours	\$10	Diagnostic
Latex	\$6	---	\$8 (10 minutes)	24–48 hours	\$14	Diagnostic
Catalase	Negligible	---	Negligible	24–48 hours	---	Diagnostic
illumi-gene®	\$25	---	\$2 (2 minutes)	55 minutes	\$27	Diagnostic
* Hourly rate based on Medical Laboratory Observer and Bureau of Labor 3/12/13 economic news release. ** Material cost based on PCMC Equipment Supply Database.						

Table 6: Labor and Materials Costs: Individual Cases

Testing Protocol (Hands-On Minutes per Case)	Number of Cases	Cost per Case	Total Cost
(A) Rapid antigen, primary culture (8 minutes per case)	286	\$8	\$2288
(B) Rapid antigen, primary culture, subculture, PYR test (25 minutes per case)	70	\$25	\$1750
(C) Rapid antigen, primary culture, subculture, PYR test, latex (35 minutes per case)	12	\$39	\$468
(total minutes 4458) Totals	368	---	\$4506
Average material/labor cost per case overall for antigen and culture = \$13			
Average material/labor cost per case overall for **illumi**gene® = \$27			

antigen negative cases that were culture positive were most expensive (C) to process at $39 per case.

For our data, the average OSOM® rapid antigen/routine culture cost per case was $13. Mean case cost for **illumi**gene® was $27. Allowable reimbursement rates from a sampling of insurance companies in our area revealed allowable reimbursement for rapid antigen/culture for Group A Strep pharyngitis to be around $18, while that for **illumi**gene® was $34 (James, 2014). According to the PCMC Cost Estimation Department, billing for OSOM® rapid antigen test/routine culture was $33 and for **illumi**gene® $49 (J.A. Daly, personal communication, January, 2014). See Table 7. Data were also obtained from Meridian Bioscience's Group A Strep Financial Analysis (Meridian Bioscience, 2014). See Table 8. In this projection, test costs for all three methods were less than the corresponding costs on the Simplified Cost Calculation (Table 7) using our study data, and the allowable reimbursement rate for culture was lower while that for **illumi**gene® was higher. Interestingly, for one large local hospital corporation, the average cost in 2013 for an average OSOM® antigen test was actually $17 with corresponding allowable reimbursement maximum of $13 and average individual culture case cost of $10 with corresponding allowable reimbursement maximum of $5. The **illumi**gene® Group A Strep DNA Assay was approved by the FDA for use beginning in 2013, and no figures are yet available in this database for its actual cost, although the allowable reimbursement rate associated with this test is $34. See Table 9.

No capital expense is required for either **illumi**gene® or OSOM® rapid antigen testing. Culture requires use of an incubator. Molecular testing requires use of a hood. The incubator and hood, as well as minor incidental costs, such as pipette tips and gloves,

Table 7: Cost, Billing, and Reimbursement Comparison – Simplified Cost Calculation

Test Method	CPT	Simplified Cost Calculation	Allowable Reimbursement	Net	Billing Amount
OSOM® antigen	87880	$4	$13	+$9	$33
Routine Culture	87081	$9	$5	-$4	
illumigene® assay	87651	$27	$34	+$7	$49

Table 8: **illumi**gene® Group A Strep Financial Analysis – Meridian Bioscience, Inc.

Test Method	CPT	Projected Cost (Meridian)	Allowable Reimbursement	Net	Billing Amount
OSOM® antigen	87880	$2	no data	no data	$33
Routine culture	87081	$0.50	$4	+$3.50	
illumigene® assay	87651	$21	$38	+$17	$49

are shared with other assays run in our microbiology laboratory. Regarding controls and repeat testing, **illumi**gene® requires positive and negative controls be run on every new lot number, as does OSOM®. These costs are considered minimal and comparable for both tests, and calculations for their inclusion are not provided here.

Taken as a whole, the characteristics of sensitivity, specificity, and accuracy, as well as convenience, speed, and economy of the assays were ranked from least desirable (1+) to most desirable (3+). See Table 10. OSOM® identified 73.1% of positive cases (28 of 104 positives missed) and culture identified 77% (23 of 104 positives missed) for an average sensitivity for the two tests combined of 75%. Sensitivity of **illumi**gene® was 99%. OSOM®, culture and **illum**igene® had almost identical

Table 9: Cost, Billing, and Reimbursement Comparison – Actual Cost 2013

Test Method	CPT	Actual cost 2013	Allowable Reimbursement	Net	Billing Amount
OSOM® antigen	87880	$17	$13	-$4	$33
Routine Culture	87081	$10	$5	-$5	
illumigene® assay	87651	no data	$34	no data	$49

Table 10: Overall Comparison of OSOM®/Culture versus **illumi**gene®

Test	Sensitivity	Specificity	Accuracy	Economy	Convenience	Speed
OSOM®/ Culture	+	+++	+	++	++	+
Illumi- gene®	+++	+++	+++	++	++	+++
Overall Desirability of Test Protocols OSOM®/culture: 52% **illumi**gene®: 91%						
+++ more desirable ++ no difference + less desirable						

specificity. Overall accuracy for **illumi**gene® was over 99%, and that of OSOM®/culture was around 93%. Average cost of OSOM®/culture testing was less than that of **illumi**gene® on our raw data assessment, but was more expensive in the actual cost scenario than its corresponding reimbursement. Since actual cost for **illumi**gene® for 2013 is not available at this time, comparison of actual cost with that of rapid antigen/culture could not be evaluated. Overall convenience as perceived by assay operators was the same for **illumi**gene® and for OSOM®/culture. Hands-on versus hands-free time was better for **illumi**gene® than OSOM®/culture. Time from receipt to result was much better for **illumi**gene® than for OSOM® antigen/culture testing.

DISCUSSION

The goal of this study was to compare traditional rapid antigen and culture methods for diagnosis of Group A Strep pharyngitis from throat swabs with the newly FDA approved **illumi**gene® rapid molecular test for Group A Strep. As noted in the results of the study, **illumi**gene® performed well on all of the characteristics evaluated. At first glance, with culture as our gold standard, **illumi**gene® appeared to have the same overall accuracy as OSOM®. On closer examination of the results, however, **illumi**gene® had a superior sensitivity (84.2% versus 97.6%), missing fewer than one Group A Strep case in 100, compared to OSOM®'s 16 missed cases per 100. When our perspective shifted to use of the alternate nucleic acid method as gold standard, results were more dramatic. OSOM® had 73.1% sensitivity, culture 76.9%, and **illumi**gene® 99.0%, with respective accuracy of 90.5%, 92.9%, and 99.5%. Culture, traditionally the gold standard for diagnosis of Group A Strep pharyngitis, missed 23 cases of all Group A Strep-positive cases. The importance of improved sensitivity lies in the fact that one missed diagnosis of Group A Strep pharyngitis can result in sequelae such as rheumatic fever and its complications, as well as invasive forms of Group A Strep disease. A test that accurately diagnoses 99% of Group A Strep-positive cases compared to a method identifying only 76% is an appealing characteristic that favors this molecular method.

A number of factors could explain the difference in accuracy across the three tests. Rapid OSOM® antigen testing historically has an accuracy rate against culture

varying from 94–99% (Veasy et al., 1987); however, under realistic conditions, using our standard culture protocol, OSOM® did not fare so well. This may be due to issues unrelated to the assay itself, such as improper sample collection or use of other than the recommended swab type. Discrepancies relative to culture could also be due to the fact that our culture protocol does not include use of Todd-Hewitt broth or SXT plates, two enhancement agents for the growth of the Group A Strep organism that have been used in validation studies for OSOM® testing; this may account for some of the false positives on OSOM® testing. The redeeming characteristics of OSOM® are that it is CLIA-waived, relatively inexpensive, and does not require viable live organism to produce valid results. This screening test provides a result within less than 15 minutes with 73.5% accuracy. It is used as a point of care test and in a health care environment remote from a clinical laboratory will likely continue to be the first line of screening for Group A Strep pharyngitis. In fact, Meridian recognizes this fact and now sponsors a "Best of Both Worlds" campaign wherein for every two **illumi**gene® kits purchased, Meridian provides three rapid antigen detection kits free of charge (Meridian Bioscience, 2014).

It is difficult to define an average sensitivity and accuracy for culture itself because routine culture is not standardized. The literature supports an "average" sensitivity rate for bacteriologic culture of around 90–95% (Kellogg, 1990) with a 10% rate of error for swab collection variability. This level of sensitivity was not supported by our data, which showed sensitivity of culture to be 76.9%. Culture is fairly inexpensive, but requires live organism for successful subculturing, and even the most cursory diagnostic procedure cannot provide a result for at least 24–48 hours after swab receipt. Additionally, culture interpretation requires a medical laboratory scientist or

microbiologist. One characteristic of Group A Strep that may explain some missed diagnoses on culture is that 1% of Group A Strep does not produce beta hemolysis, one of the key identifiers on culture plates for diagnosis in the clinical laboratory (James & McFarland, 1971). Also, patients who have taken antibiotics may indeed be suffering from Group A Strep infection, but antibiotics render the organism nonviable very rapidly, and thus unculturable. Finally, quantity of Group A Strep in the sample affects its detectability, and obscuration by normal flora may limit visualization of minute quantities of Group A Strep on a culture plate. Culture requires a minimum of 100,000 colony-forming units of organism for visual detection, while **illumi**gene® can detect as little as 400 colony-forming units. (Meridian Bioscience, 2012).

illumigene®, with its superior accuracy and sensitivity, does not require viable organism for testing, but does require processing by a medical laboratory scientist and takes about 50 minutes from swab receipt to diagnosis. The **illumi**gene® assay can be affected by zincum aceticum or zincum gluconicum-containing cold remedies such as Zicam, although there is no data clarifying for how long after use of Zicam the test result is affected. Patients with a history of recent use of these agents are not candidates for **illumi**gene® testing until further research elucidates the duration of zincum interference. Anecdotally, however, the effects of zinc on the **illumi**gene® assay are believed to resolve within an hour of administration (L. Reid, Personal Communication, April, 2014). Finally, **illumi**gene® results could be impacted by the presence of an aberrant sequence of the *Streptococcus pyogenes* SpeB gene, a specific segment of which it must detect to produce positive results. Either of these factors may be responsible for the single false negative case on **illumi**gene® testing in our data. One downside of testing only on

nonviable organisms is that they cannot be subsequently cultured and saved for research purposes.

A common complaint about some laboratory tests, both screening and diagnostic, is that they are too sensitive, and it is impossible to distinguish carrier status from infection status using these tests. Since swab testing occurs while sore throat symptoms are present, this is a difficulty that cannot be solved by the test itself. The only true test of Group A Strep pharyngeal infection is the detection of antibody response to the infection, the production of which is the culprit that can cause damage in rheumatogenic cases. A patient is at risk for sequelae if not treated before antibody production begins, but waiting for the detection of antibody production to "prove" infection puts the patient at risk for these sequelae, so prevention of sequelae must take the form of astute clinical assessment while the patient is symptomatic, with patients suspicious for Group A Strep pharyngitis having their throats appropriately swabbed and the sample sent for confirmatory testing, followed by the administration of antibiotics in positive cases. Thus the algorithm for reliable diagnosis must include both discerning clinical evaluation and trustworthy laboratory testing that is rapid enough to obviate the need for empiric therapy. Clinical guidelines for diagnosis vary depending upon many factors, but the Centor score algorithm, devised to differentiate clinically between viral and bacterial pharyngitis, was structured to minimize this clinical uncertainty, and could be considered by clinicians as a useful algorithm for more accurate clinical diagnosis. See Appendix E. There is still the possibility that patients carrying detectable Group A Strep but suffering from viral pharyngitis may be erroneously treated with penicillin, but insightful clinical evaluation coupled with appropriate confirmatory testing should minimize this problem,

and this error pales in comparison to the 23 children suffering with Group A Strep pharyngitis that went undiagnosed in our data, children who were likely not treated and now carry the risk of falling victim to rheumatic fever or invasive Group A Strep infection.

Molecular assays also have the reputation for being "too hands on" and time consuming and can be fraught with contamination problems. Some molecular assays require transfer of molecular material from one open tube to another, and then to another, with intervening, time-consuming steps that need to be done within a precise time frame. Many molecular procedures require that each step be conducted in a progressively less contamination-prone environment. These difficulties are minimized with the **illumi**gene® assay. As illustrated in Appendix C, **illumi**gene® swabs are enclosed within a lysis tube. Drops from this closed lysis tube are expelled into a heat-treatment tube. Finally, this tube is opened, and small volumes are pipetted into a dual-chamber tube. One of these chambers is an internal control; the second is the sample reaction chamber. This tube arrangement minimizes the potential for contamination and also eliminates the need for extra controls being included on each run, as they are integral to each test device. Positive and negative controls are required only with each new kit or lot number. All steps of this procedure can be performed under the same hood. Processing external to the hood occurs either in the 95 ^{o}C heat block or the **illumi**pro-10^{TM} instrument.

Since **illumi**gene® is an isothermal reaction, it does not require as much time as traditional PCR methods, and hands-on processing time is less than 3 minutes. A beneficial characteristic of this assay is that once components are placed on the

illumipro-10™ reader, the technologist can walk away. When the assay is finished, the instrument prints the result and shuts itself down without attention by the technologist. Although on our questionnaire for operators of **illumi**gene® and culture, half of the respondents reported problems with invalid results, this observation was not borne out in our study. Only two of our 368 cases required repeat because of invalid results. Result options on the **illumi**pro-10™ instrument are positive, negative or invalid, and are otherwise unequivocal. A new set of samples can be started on the **illumi**pro-10™ instrument every 36 minutes, so on-demand testing to meet 1-hour turnaround time is feasible within the constraints of our data set.

One definite advantage of the **illumi**gene® system is that at present each standalone **illumi**pro-10™ instrument can accommodate testing for five different assays —Group A Strep, Group B Strep, *Mycoplasma pneumoniae*, *Clostridium difficile,* and *Bordetella pertussis*. Meridian is currently working on approval for more assays that will employ this instrument. The **illumi**pro-10™ instrument itself is flexible, also. The procedure is very similar for the five FDA-approved assays. Each **illumi**pro-10™ instrument has two test drawers that will each accommodate five tests at a time, and a separate test can be run in each drawer simultaneously. With this instrument supporting five different assays in the clinical laboratory, its value will increase as test protocols change to include it.

Molecular tests are historically expensive, so the issue of relative expense for all three test methods was a subject that required scrutiny in this study and unfortunately could not be unequivocally resolved. On cursory analysis, material and labor costs for **illumi**gene® are twice those for the combination of OSOM® and routine culture, but

even very careful labor and material cost evaluation for our data set was far from reflective of real world costs, at least for rapid antigen and culture testing. Thus, it is important to consider not only costs of these assays but also the other relevant characteristics of these tests, as elucidated above, with particular mindfulness to the overall benefit to the welfare of our patients. Attempts to accurately predict test costs prospectively in the health care environment in which we now find ourselves is difficult at best, as is borne out by the contrast between allowable reimbursement and actual cost of the rapid antigen and culture tests costs from 2013 discussed above. Looking at the stunning discrepancy between actual 2013 test costs that are roughly 150% of allowable reimbursement rates, one is compelled to contemplate how long a health care entity can continue to offer testing that it is essentially paying its patients to undergo. In the current health care environment, with actual and projected costs so difficult to ascertain, illumigene® is, at the very least, a competitive contender and deserves the opportunity to prove itself in the clinical setting.

Each and every case of rheumatic fever is, by definition, preceded by a case of Group A Strep pharyngitis. Thus, a single undiagnosed case of Group A Strep pharyngitis can result in rheumatic fever with concomitant financial costs averaging more than $13,000 annually for medical care, lost parental wages, and absenteeism (Terreri et al., 2001). One undiagnosed case of GAS pharyngitis can also result in invasive streptococcal infection, with an average cost in children of over $10,000 per case annually (Black & Shinefield, 2002); thus, a discussion of cost must include that to the patient if a case of Group A Strep pharyngitis is not accurately diagnosed. Group A Strep pharyngitis treated immediately with intramuscular penicillin or oral penicillin, while sore throat is

still present, before the acute infection self-limits in about 5 days, has beneficial implications for the patient in terms of more rapid restoration of health, reduced infectivity, and reduced chance of developing sequelae. On the other hand, while Group A Strep remains exquisitely sensitive to penicillin, judicious use of this antibiotic is very important for maintaining the patient's microbiome. Our question must be, "What is the best course of action for our patients?" Do we continue to use less expensive tests with reduced sensitivity and run the risk of subsequent infections, do we prescribe penicillin empirically, or do we look at all of the characteristics of the resources at our disposal and choose that which is the best for optimal patient outcome?

For several decades, with advancements in therapies, procedures, and technology, medical costs have risen at an alarming rate, impacting both patients and health care systems. Several hospital corporations (Cosgrove et al., 2013; James & Savitz, 2011) have studied how to contain these costs and have implemented changes in their approach to health care management, with the direct goal being that of improving treatment and patient outcomes and indirectly to contain costs by shifting focus from provider to process variation. One such health care company has, through a feedback-loop protocol between evidence-based medicine algorithms, patients, and their providers, introduced a mechanism whereby protocols are introduced then modified by provider input relative to individual patient care. The providers supplying the input as "teachers" in this system, helping to modify and make more efficient the treatment algorithm, are also the "learners" in this system since the experience and input from a wide range of providers is gathered, collated, "fed back" into the system, and shared with all providers, and the protocol is modified accordingly. This approach is called "shared baselines," and an

example of this feedback loop strategy was used to implement modification of and improvement in existing guidelines for labor induction by defining precisely via evidence-based indicators when induction was medically appropriate, collecting provider variations to this practice, collating variations and modifying guidelines, and deploying this information back throughout the entire system. The result, after several months of using this feedback loop, was to reduce the variation in physician practices, reduce the number of elective labor inductions from 28% of deliveries to less than 2%, and reduced health care costs in Utah by about $50 million annually. This technique was not implemented specifically to save money, but to directly improve efficiency and enhance patient care. A side-effect of the success of this change in treatment algorithm was the reduction in health care cost (James, 2011). Thus, perhaps inadvertently and unexpectedly, in the process of tweaking the existing treatment algorithm to improve patient outcome, cost was reduced. W. Edwards Deming, father of quality improvement, argued that most process changes that produce better physical outcomes also cause costs to fall (Deming, 1986), substantiated by the above example and by many others.

As the trend continues toward the popularity of health care delivery groups, which are allotted a flat fee per month for patient care and are expected to use these funds to provide optimal care within these financial constraints, **illumi**gene® is presented as a potential participant in new algorithms aimed at improvement in overall patient health and well being. All of the characteristics of **illumi**gene® described in this text portend improvement of patient care and outcomes, and if Deming's adage holds true, inadvertently and perhaps unexpectedly, the beneficial side-effect of reduced overall health care cost in the control of Group A Strep pharyngitis, and thus its serious sequelae, will result.

CONCLUSIONS

It is clear from the above data that the **illumi**gene® Assay for Group A Strep pharyngitis is both dependable and timely, fulfilling the value criteria for clinicians. After resolution of discrepant results in our study, **illumi**gene® was proven to be a remarkably sensitive and specific assay, more accurate than either traditional method of testing, alone or combined. **Illumi**gene® performed very well in all aspects evaluated in this study and deserves the opportunity to prove itself as a valuable addition to the repertoire of the clinical laboratory. If given this opportunity, **illumi**gene® will undoubtedly become a key player in efficient, cost-effective improvement in the diagnosis, treatment, and outcome of our patients afflicted with Group A Strep pharyngitis and will fulfill Deming's prediction of concomitant reduction in associated health care costs.

APPENDIX A

OSOM® ULTRA STREP A TEST

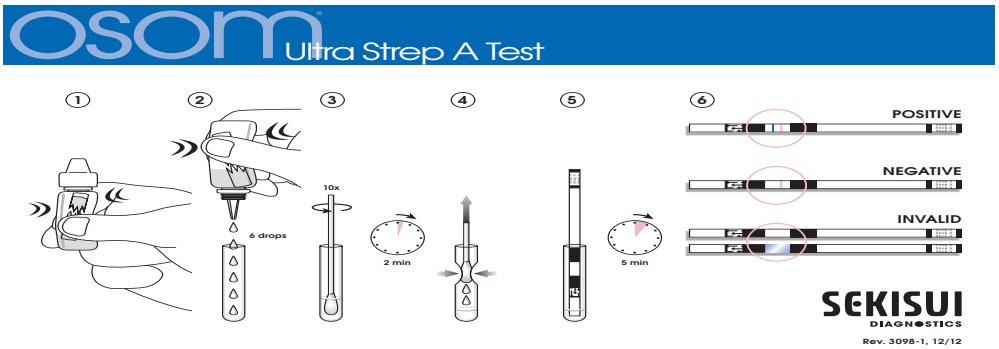


Figure A.1. The OSOM® Ultra Strep A Test is a CLIA-waived color immunochromatographic assay for the rapid qualitative detection of Group A Strep antigen directly from throat swabs. Viable and nonviable organisms can be detected within 7 minutes. The throat swab is subjected to chemical extraction of carbohydrate antigen specific to Group A Strep. The test strip, a lateral flow device coated with antibody-labeled color particles in two locations, is then placed in the extraction solution. Liquid migrates up the test strip. If Group A Strep carbohydrate antigen is present, it complexes with the labeled antibody impregnated in the strip (and eluted by the liquid). These complexes will bind the anti-Group A Strep capture antibody and a blue "positive" line will be visible. An internal control will also be visible when nonspecific antigen in the sample binds the nonspecific antibody impregnated in another section of the strip. This red line confirms the validity of the test. The illustrated procedure depicts the following: (1) Obtain refrigerated reagent kit. Bring reagents to room temperature. (2) Dispense 6 drops of reagent into test tube. (3) Agitate patient throat swab in reagent tube vigorously, and allow it to rest in tube for 2 minutes (4) Remove swab from reagent tube and insert lateral flow device into reagent/patient sample tube. (5) Incubate at room temperature for 6 minutes. (6) Remove lateral flow device from reagent/patient sample tube and read results.

APPENDIX B

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

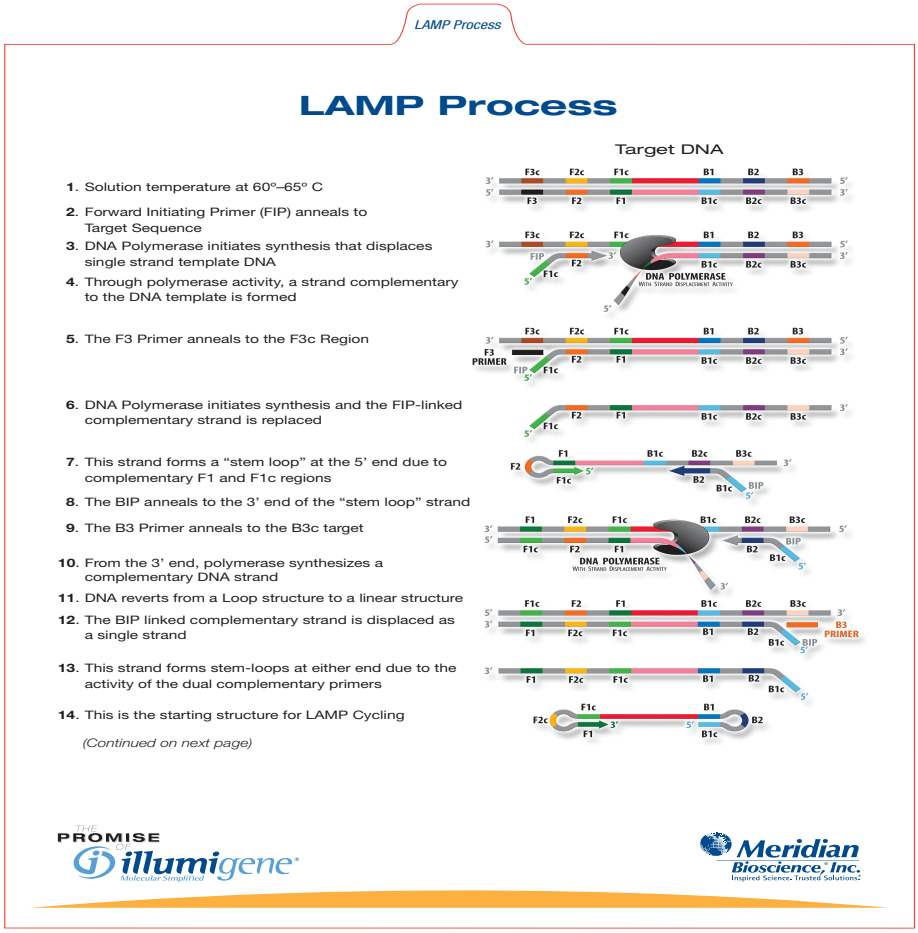
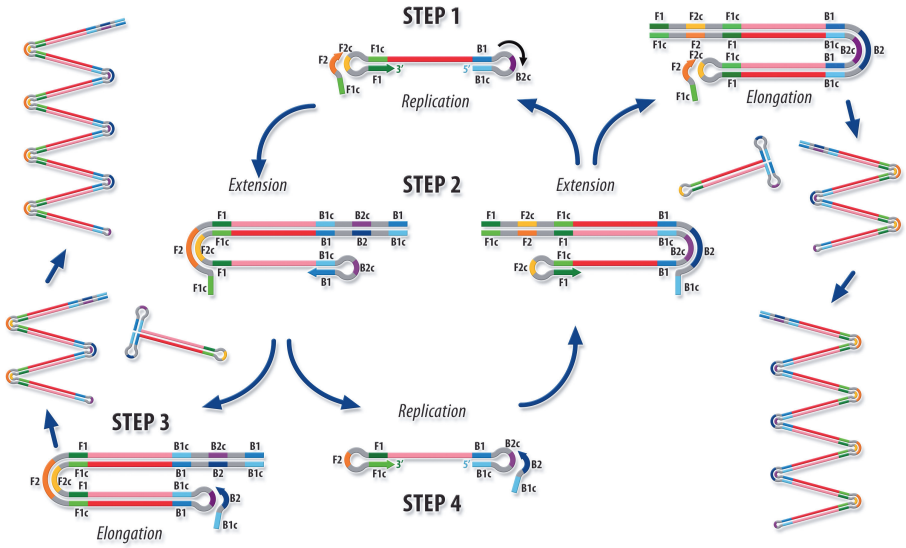


Figure B.1. In this isothermal nucleic acid amplification method, several primers and a strand-displacement polymerase initiate the amplification process, and are augmented by loop primers which enhance the rapidity and abundance of amplification, the result of which is magnesium pyrophosphate, a compound causing turbidity to the reaction components. Detection of this turbidity implies the presence of Group A Strep DNA in the reaction chamber.

LAMP Process

illumigene® Molecular Diagnostic System

LAMP Process (cont.)



15. LAMP Cycling. A dumbbell-like DNA structure is quickly converted into a stem-loop DNA by self-primed DNA synthesis. FIP anneals to the single stranded region in the stem-loop DNA and primes strand displacement DNA synthesis, releasing the previously synthesized strand. This released single strand forms a stem-loop structure at the 3' end because of complementary B1c and B1 regions.

Then, starting from the 3' end of the B1 region, DNA synthesis starts using self-structure as a template, and releases FIP-linked complementary strand (STEP 2).

The released single strand then forms a dumbbell-like structure as both ends have complementary F1–F1c and B1c–B1 regions, respectively (STEP 4).

This structure is the 'turn over' structure of the structure formed in STEP 1. Similar to the STEPS 1 THROUGH 4, structure in STEP 4 leads to self-primed DNA synthesis starting from the 3' end of the B1 region.

Furthermore, BIP anneals to the B2c region and primes strand displacement DNA synthesis, releasing the B1-primed DNA strand. Accordingly, similar structures to STEPS 2 AND 3 as well as the same structure as STEP 1 are produced. With the structure produced in STEP 3, the BIP anneals to the single strand B2c region, and DNA synthesis continues by displacing double stranded DNA sequence. As a result of this process, various sized structures consisting of alternately inverted repeats of the target sequence on the same strand are formed.

For more information, contact an *illumigene*™ specialist at 1-888-763-6769 or visit us on the web at www.meridianbioscience.com.

THE PROMISE OF illumigene® Molecular Simplified

Meridian Bioscience, Inc. Inspired Science. Trusted Solutions.

Figure B.1. Continued

APPENDIX C

ILLUMIGENE® GROUP A STREPTOCOCCUS DNA AMPLIFICATION ASSAY

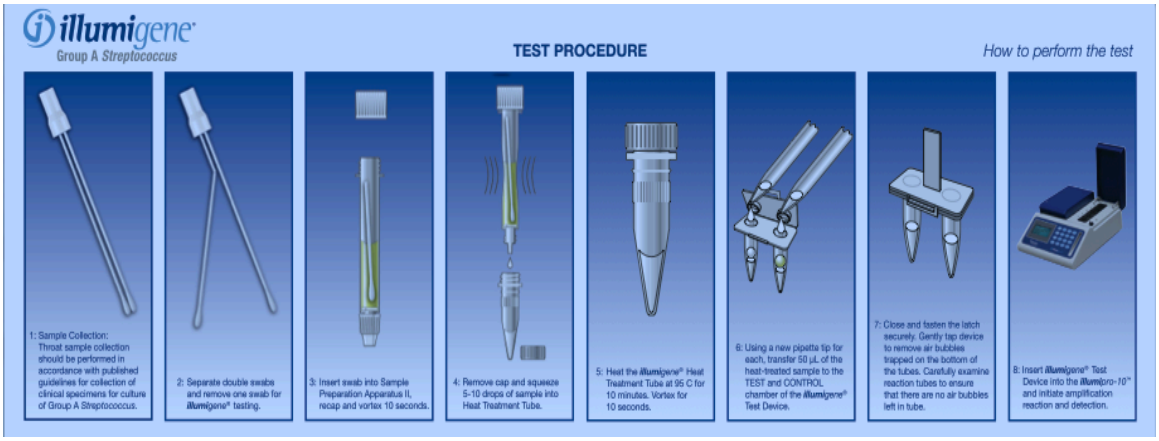


Figure C.1. The illumigene® Group A Streptococcus DNA Amplification Assay is a qualitative test for Group A Strep antigen from throat swabs. This loop-mediated isothermal DNA amplification technology (LAMP) uses specific primers to target a highly conserved 206-base-pair region of the *Streptococcus pyogenes* genome residing in the pyrogenic exotoxin B (SpeB) gene. Patient swab is inserted into a lysis solution that contains formalin-treated *Escherichia coli* containing *Staphylococcus aureus* DNA. Some of this solution is transferred into a heat-treatment dilution tube and incubated at 95^{o}C for 10 minutes. The test device has one test chamber containing Group A Strep-specific primers and one control chamber containing *Staphylococcus aureus*-specific primers. This control chamber functions as an internal control validating the test result by monitoring amplification inhibition, assay reagent performance and sample processing effectiveness. Control *Staphylococcus aureus* must be amplified and detected to have the test considered valid. Detection is not via the amplification itself but a by-product of the reaction, magnesium pyrophosphate, which is a white precipitate that produces a turbidity to the reaction solution. Change in sample absorbance resulting from precipitation of magnesium pyrophosphate indicates the presence of Group A Strep and is reported as positive. Lack of detectable change in absorbance is reported as negative. The illustrated procedure depicts the following: (1) Obtain patient swab; bring reagent kit to room temperature. (2) Place one swab in the lysis tube. (3) Vortex. (4) Squeeze drops into heat treatment tube. (5) Place heat treatment tube into 95^{o}C heat block for 10 minutes. (6) Vortex heat-treated tube, then pipette 50 microliters of this sample to the test device "test" and "control" compartments. (7) Close reaction device and tap to remove all air bubbles. (8) Insert reaction device into illumipro-10^{TM} device and initiate amplification.

APPENDIX D

OPERATOR ASSESSMENT OF ASSAY PERFORMANCE CHARACTERISTICS

Name _____

Operator Survey of the Performance of **illumi**gene® Group A Streptococcus DNA Amplification Assay Versus Routine Culture for Group A Streptococcus Pharyngitis

Please rank the **illumi**gene® Group A Streptococcus DNA Amplification Assay and Routine Culture for Group A Streptococcus Pharyngitis based on your experience with both. Rank each statement on a scale of 1+ to 4+ as below:

1+ least convenient 2+ less convenient 3+ more convenient 4+ most convenient

1. The training time required for competent use of the **illumi**gene® Group A Strep Assay is _____.
2. The training time required for competent use of Routine Culture for GAS is _____.

3. Time required for definitive diagnosis of GAS with the **illumi**gene® assay is _____.
4. Time required for definitive diagnosis of GAS with Routine Culture for GAS is _____.

5. Procedural complexity of the **illumi**gene® assay is _____.
6. Procedural complexity of Routine Culture for GAS is _____.

7. Number of steps required for the **illumi**gene® assay is _____ .
8. The number of steps required for Routine Culture for GAS is _____.

9. Hands-on time required by technologist for the **illumi**gene® assay is _____.
10. Hands-on time required by technologist for Routine Culture for GAS is _____.

11. Ease of interpretation of **illumi**gene® is _____.
12. Ease of interpretation of Routine Culture for GAS is _____.

13. Need for repeat testing on **illumi**gene® to obtain valid result is _____.
14. Need for repeat testing on Routine Culture for GAS to obtain valid result is _____.

15. Overall convenience of **illumi**gene® assay is _____.
16. Overall convenience of Routine Culture for GAS is _____.

Figure D.1. Operator Assessment of Assay Performance Characteristics: **illumi**gene® Versus Culture

APPENDIX E

CENTOR SCORES FOR CLINICAL MANAGEMENT OF PHARYNGITIS

Table E.1. Centor Scores for Clinical Management of Pharyngitis

Criteria	Points
Absence of cough	1
Tonsillar exudate or swelling	1
Swollen cervical lymph nodes	1
Temperature elevated to 38^{O} C	1
Patient age 3-14 years	1
Patient age 15-44 years	0
Patient age greater than 45 years	-1

Used widely in Europe, Centor scores have not been as popular in the United States. Using the above algorithm, a child of 13 years presenting with tonsillar exudates and fever would have a Centor score of 3. According to the American Society for Internal Medicine, Centor scores of 2–3 are recommended to have rapid antigen detection testing, with throat cultures reserved only for children. A Centor score of 4 warrants treatment without confirmatory testing, but this assessment alone results in antibiotic treatment of 50% of negative cases (Centor, Allison & Cohen, 2007).

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